STUDY ON THE PREVALENCE, SPECIATION AND ANTIBIOGRAM OF ENTEROCOCCI ISOLATED FROM HETEROGENOUS CLINICAL SAMPLES

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BONAFIDE CERTIFICATE

This is to certify that this dissertation entitled **"STUDY ON THE PREVALENCE, SPECIATION AND ANTIBIOGRAM OF ENTEROCOCCI ISOLATED FROM HETEROGENOUS CLINICAL SAMPLES"** is a bonafide record work done by **Dr. LAVANYA. T. KAMALASEKARAN** under my direct supervision and guidance, submitted to the Tamil Nadu Dr. M.G.R. Medical University in partial fulfillment of University regulation for MD, Branch IV –Microbiology.

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DECLARATION

I **Dr. LAVANYA. T. KAMALASEKARAN** solemnly declare that this dissertation titled **"STUDY ON THE PREVALENCE, SPECIATION AND ANTIBIOGRAM OF ENTEROCOCCI ISOLATED FROM HETEROGENOUS CLINICAL SAMPLES"** has been done by me. I also declare that this bonafide work or a part of this work was not submitted by me or any other for any award, degree, diploma to any other University board either in India or abroad.

This is submitted to The Tamilnadu Dr. M. G. R. Medical University, Chennai in partial fulfillment of the rules and regulation for the award of Doctor of Medicine degree Branch –IV (Microbiology) to be held in March 2008.

Place : Madurai **Dr. LAVANYA.T. KAMALASEKARAN**

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INTRODUCTION

Enterococci are Gram positive, facultative anaerobic organisms that were previously considered to be of the genus streptococci (Group D). Later they were found to have different nucleic acids by hybridisation technique and were separated into their own class in 1984.

Enterococci comprise a significant portion of the normal flora of the gastrointestinal tract with some also being found on the skin in oropharyngeal and vaginal secretions and in perineal area[103]. Enterococcal infections are most commonly found in urinary tract infections (16%) and 8% of all bacteremias. Endocarditis, meningitis, neonatal sepsis, respiratory infections are less commonly attributed to enterococcal organisms[103]. Most human isolates are due to either E. faecalis (74-90%) or E .faecium (5-16%). Occasionally, human infections can be due to Enterococcus raffinosus, Enterococcus casseliflavus, Enterococcus durans, or Enterococcus avium[55].

Enterococci are now prominent as one of the emerging nosocomial pathogens, ranking second only to E.coli in total nosocomial infections accounting for more than 12% of all cases. The problem of nosocomial enterococcal infection is compounded by multiple antibiotic resistance. Their resistance to commonly used antimicrobial agents and the ease with which they appear to attain and transfer resistant genes, give rise to enterococci with high level aminoglycoside resistance, β lactamase production & glycopeptide resistance [103].

Over the past 2 decades there has been an increasing number of reports of Enterococcus species with induced resistance to multiple antibiotics, and therapeutic options have become increasingly limited. The first evidence of high-level resistance of Enterococcus species to streptomycin and gentamicin (minimum inhibitory concentration [MIC] > 2,000 μg/L) was documented in the 1970s and during the 1980s the prevalence of these resistant strains increased dramatically in several locales in North America and Europe. High-level aminoglycoside resistance eliminates the option of using aminoglycosides in combination with cell-wall active agents (e.g., penicillin or ampicillin) for synergistic activity.Resistance to ampicillin is being observed with increasing frequency and may be due to a decreased ability to bind to penicillins or to the production of β lactamase by the microorganism.

The development of resistance to vancomycin, which is potentially much more problematic, was first reported in Europe in 1986.Since then, outbreaks of Vancomycin resistant enterococci (VRE) infections have been described in several institutions and other health setting in the United States. The mechanisms of resistance to vancomycin have been described,but the concern from a clinical perceptive is the loss of vancomycin and other glycopeptide antibiotics for the treatment of serious enterococcal infections. With an increasing incidence of Enterococcus species resistant to both penicillins and aminoglycosides, the addition of vancomycin resistance would severely limit therapeutic options. The vancomycin resistance trait in Enterococcus species is transferable, and perhaps the greatest threat of VRE is the potential emergence of vancomycin resistance in methicillin-resistant Staphylococcus aureus .

The clinically important Enterococci are E.faecalis and E.faecium. In these species, vancomycin resistance is associated with the Van A, Van B Genes, Van D or Van E gene cluster. Van A and Van B genes are acquired through the transfer of plasmids or transposons. The VanA phenotype is highly resistant to both vancomycin (MIC of 64 µg/mL or more) and to teicoplanin, the investigational glycopeptide (MIC of 16 µg/mL or more). The Van B phenotype shows moderate to high-level resistance to vancomycin (MIC of 32 to 256 µg/mL) but usually remains susceptible to teicoplanin (MIC of less than 1 µg/mL). These two phenotypes are the most prominent and are seen primarily in E faecium, but they also occur in E faecalis. A third phenotype, VanC, shows low-level resistance to vancomycin (MIC of 8 to 32 µg/mL) without teicoplanin resistance; this phenotype is seen primarily in *E gallinarium* and *E casseliflavus*. Vancomycin resistance occurs when proteins are synthesized by the resistant enterococci, called "VanA," "VanB," and "VanC." These proteins produce resistance by acting as ligases that alter the cell-wall precursors, which are the targets of vancomycin. E.gallinarum and E.casseliflavus possess intrinsic, non transferable vancomycin resistance encoded by Van C_1 and Van C_2 ligase genes respectively. These species rarely cause infections and are associated with transmission and hospital outbreak. Hence, for infection control practices and prevention of person to person transmission ,detection of Vancomycin resistant Enterococci and speciation is necessary. High Level Aminoglycoside resistance (HLAR) has been observed in E.faecalis and E.faecium.

Few virulence factors have been identified like haemolysin, gelatinase, aggregation substances and surface protein. Haemolysin increases the virulence of E.faecalis in infection models of different animal species. Gelatinase producing strains resulted in more severe clinical findings in experimental endocarditis model. Aggregation substances, is a surface protein encoded by sex-pheremone responsive plasmids, increases the number of bacteria adhering to renal and intestinal epithelial cells, suggesting aggregation substances is important for colonisation and translocation of host tissues by E.faecalis[66].

Enterococcal surface protein was found in E.faecalis, strain that caused multiple infections within a hospital ward. A variant Enterococcal surface protein gene was also found in Vancomycin Resistant Enterococcus faecium spreading in hospitals [41].

Enterococci account for as many as 10% of cases of neonatal bacteremia and septicemia. Incidence of neonatal enterococcal septicemia increased from 0.12 per 1000 live births in 1982 to 0.8 per 1000 live births in 1986. Enterococcus may cause early-onset (within 7 days of birth) or late-onset (>7 days) neonatal sepsis. Early-onset sepsis caused by enterococci is milder than that caused by group B streptococci. Most cases of enterococcal bacteremia in neonates are nosocomial. Central venous catheters, necrotizing enterocolitis, and intra-abdominal surgery are risk factors. Enterococcus may cause focal skin and soft tissue infections, meningitis, and conjunctivitis in the neonate. Most neonatal infections are caused by E. faecalis[55].

Neonatal septicemia remains one of the most important causes of mortality despite considerable progress in hygiene, introduction of new antimicrobial agents, and advanced measures for early diagnosis and treatment .Group B streptococcal disease is the most important cause of neonatal sepsis in Europe and North America,but there is a preponderance of Gram-negative organisms in tropical and developing countries.As neonatal septicemia is a life-threatening emergency and delay in diagnosis and treatment with appropriate antibiotics may have devastating consequences and hence surveillance is needed to identify the common signs and pathogens of neonatal septicemia as well as the antibiotic sensitivity patterns for the agents of septicemia in a particular area[3].

Clinical presentations of patients with culture-proven serious neonatal bacterial infection were respiratory distress (47%), lethargy (40%), jaundice (40%), fever (36%) and poor feeding (27%). Respiratory distress is significantly more common in early-than late-onset septicemia. Males have been reported to be 2 to 5 fold more likely than females to develop septicemia. History of unclean vaginal examination was associated with a 10% incidence of deep infection in one study [3].

Risk factors for acquisition of VRE and enterococcal infections include history of the following:

- Prolonged hospitalization
- Long stay in ICU
- Surgical reexploration following liver transplantation
- Prior use of antibiotics, mainly vancomycin and cephalosporins
- Immunocompromised state
- Breakdown of normal physical barriers (eg, gastrointestinal tract, skin, urinary tract)
- Neurosurgical procedures and use of neurosurgical devices [54]

As Enterococcal infections are one of the leading cause of nosocomial infection,this study was focussed on the isolation of enterococcus from the various clinical samples collected from Government Rajaji Hospital (GRH) and to speciate them and to analyse the common species responsible for infections wardwise,specimenwise,sexwise and age wise. Further the common factor responsible for the emergence of this infection was also studied.

AIM AND OBJECTIVES

- To Isolate Enterococcus from hetrogenous samples collected from GRH by culture and confirmation by biochemical tests.
- To speciate Enterococcus and to study their etiological role.
- To study the antibiogram of the isolated species.
- To Identify predisposing factors for Enterococcal infections.

REVIEW OF LITERATURE

Koneman's Textbook of Diagnostic Microbiology ,6th edition has stated that the genus Enterococcus includes the enterococcal members previously classified with the group D streptococci.These organisms are normal residents of the gastrointestinal and biliary tracts .They are becoming increasingly important agents of human disease,largely because of their resistance to antimicrobial agents to which other streptococci are generally susceptible.[20]

The term "enterocoque",was first used by **Thiercelin et al** in 1899.Thiercelin published a paper in French, 'Sur un diplocoque saprophyte de l'intestin susceptible de devenir'in which he used the term to describe the Gram positive cocci in pairs that he observed to be present in the faeces.[92,93]

In 1906, Andrews and Horder et al first used the term Streptococcus faecalis[6,27].

In 1919, Orla – Jensen et al described a second organism S.faecium which differed from the fermentation patterns of S.faecalis [64] .

In **1935 ,Sherman and wing et al** proposed a third species Streptococcus durans[84] .

In 1937, Sherman et al stated that durans was similar to S.faecalis but had less fermentation activity[84].

In 1938 ,Sherman et al used the term "enterococcal group" to describe Streptococci that grew at 10ºC and 45ºC, grew in broth with pH adjusted to 9.6 and in broth containing 6.5% NaCl and survived heating to 60ºC for 30 minutes[84,93].

In 1967, Nowlan and Deibal et al added Streptococcus avium to enterococcal group[63,93].

In 1970, Kalina et al proposed a separate genus for the Enterococcal streptococci be established and suggested that, based on cellular arrangement and phenotypic characteristics, S.faecalis and S.faecium and the subspecies of these 2 taxons be named Enterococcus[93] .

In 1984, Scheifer and Kilpper Balz et al proved that by genetic evidence like DNA-DNA and DNA rRNA hybridization studies and by 16 S rRNA studies that S.faecalis and S. faecium were different from the other members of the genus to merit a separate genus [83].

CKJ Paniker and R. Ananthanarayan, Text book of Microbiology, 7th edition has described the genus Streptococci as follows. Streptococci are first divided into obligate anaerobes and facultative anaerobes. The former are designated Peptostreptococci. The aerobic and facultative anaerobic Streptococci are classified on the basis of their haemolytic properties. Brown (1919) categorised them into three varieties based on their growth in 5% horse blood agar[13].

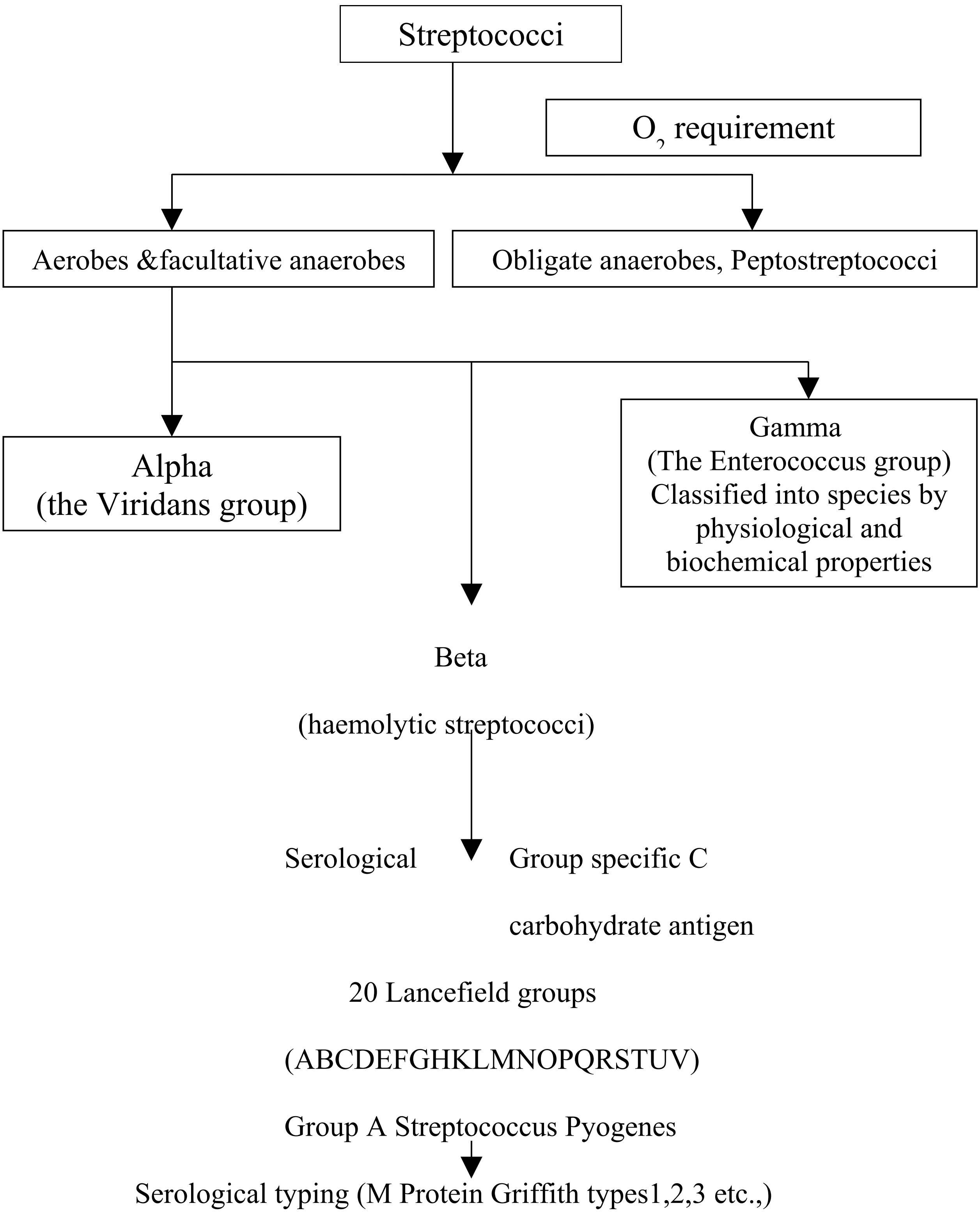
Alpha (α) haemolytic Streptococci produce a greenish discolouration with partial haemolysis around the colonies. The zone of lysis is small (1 or 2mm wide) with indefinite margins, and unused erythrocytes can be made out microscopically within this zone. They are known as "Viridans Streptococci"[13]. Beta (β) haemolytic Streptococci produce a sharply defined, clear,colourless zone of haemolysis 2-4mm wide, within which red cells are completely lysed. Most pathogenic Streptococci belong to this group[13]. Gamma (γ) or non haemolytic Streptococci produce no change in the medium and so are sometimes referred to as "Indifferent Streptococci". They include the faecal Streptococci (Enterococci, Streptococci faecalis) and selected species. They are called the "Enterococcus group"[13].

In 1933 Lancefield et al classified Haemolytic Streptococci serologically into groups based on the nature of a carbohydrate (C) antigen on the cell wall. These are known as Lancefield groups, twenty of which have been identified so far and named A-V (without I & J). Majority of human infections are caused by Group A β-haemolytic Streptococci. They may be further subdivided into types based on the protein (M,T, and R) antigens present on the cell surface (Griffith typing)[38,13].

Group D Streptococi can be classified into two groups

1) The Enterococcus group (Enterococci or faecal Streptococci) which have been reclassified as separate genus, Enterococcus containing for example

E.faecalis, E.faecium, E.durans[13]. 2) Non Enterococcal group, for example Str.bovis and Str.equinus[13].



R. R. Facklam, D. S. Sahm, and L. M. Teixeira et al in the Manual of Clinical Microbiology, has described enterococci as follows.They are Gram-postive cocci, occuring singly, in pairs, or in short chains and are facultatively anaerobic.They grow between 10ºC and 45ºC (most strains), optimum at 35ºC ,grows in broth containing 6.5% NaCl and hydrolyze esculin in the presence of 40% bile salts (bile-esculin medium).Motility was observed in some species .Hydrolysis of leucine-ß-naphthylamide (LAP) and hydrolysis of pyrrolidonyl-ß-naphthylamide (PYR) occurred with the exception of *E. cecorum, E. columbae,* and *E. saccharolyticus.*They are catalase negative and nearly all strains are homofermentative, without gas production.Glucose fermentation results in production of lactic acid.Cell wall-associated glycerol teichoic acid antigen is identified as streptococcal group D antigen[72].

According to **Huycke, et al** Enterococci normally inhabit the bowel. They are found in the intestine of nearly all animals, from cockroaches to humans.In humans, typical concentrations of enterococci in stool are up to 10^8 CFU per gram. Although the oral cavity and vaginal tract can become colonized, enterococci are recovered from these sites in fewer than 20% of cases. The predominant species inhabiting the intestine varies. Of 14 or more enterococcal species, only *E. faecalis* and *E. faecium* commonly colonize and infect humans in detectable numbers. *E. faecalis* is isolated from approximately 80% of human infections, and *E. faecium* from most of the rest. Infections to other enterococcal species are rare [28].

M.G.Karmakar et al have isolated 52 enterococci ,of which 42 were E.faecium [45].

Adam .N.Treitman et al have reported that there is an increasing emergence of enterococci over the past 10 years in their medical centre.They have stated that the percentage of enterococci identified as E.faecium has increased from 12.7% to 22.2% and the percentage of E.faecium resistant to vancomycin has gone up from 28.9% to 72.4% [4].

Shuqiu Cheng et al have stated that E.faecium is an important emerging nosocomial infection .They have devised a PCR assay for the rapid identification of E.faecium[87].

Patrick & Murray et al in the Text book of Manual of ClinicalMicrobiology, 9th edition has stated that Enterococci are considered to be the most abundant Gram-positive cocci colonizing the intestine, with *E.faecalis* being one of the most common bacteria isolated from this site. Other species, such as *E.faecium* as well as *E.casseliflavus, E.durans, and E.gallinarum*, are also found in variable proportions in the gastrointestinal tract of humans. Since the enterococci are opportunistic pathogens, the incidence of each species found in human infections probably reflects the distribution of the different species of Enterococcus in the human gastrointestrinal tract. This site is believed to represent an important reservoir for strains associated with disease; from this location they may migrate to cause infections and can also disseminate to other hosts and to the environment. Even though the same enterococcal species can be found in several different animal species, the information available on the distribution of distinct enterococcal species in other sources indicates differences from the distribution in humans [66].

Sherwood L Gorbach et al has said that Urinary tract infections are the most common type of clinical disease produced by enterococci, and urine cultures are the most frequent sources of enterococci in the clinical microbiology laboratory. In addition to uncomplicated cystitis or pyelonephritis, or both, enterococci have also been shown to cause prostatitis and perinephric abscess. Most enterococcal urinary tract infections are nosocomial and are associated with urinary catheterization or instrumentation, or both. There is strong evidence to suggest that the prevalence of nosocomial enterococcal urinary tract infections is increasing in a number of hospitals [85].

Mcneely D. F et al have reviewed neonatal enterococcal bacteremia.They have reported an increased number of these infections since the late 1970s and the increased isolation of organisms resistant to many commonly used antimicrobials . Common characteristics associated with enterococcal bacteremia included the presence of a central vascular catheter,necrotizing enterocolitis and abdominal distension[54.]

Susan L Fraser, et al have reported that Enterococci cause 5-15% of all endocarditis cases.This condition usually occurs in older patients. Their presentation is typically subacute.Usually, left-sided endocarditis and mitral valve involvement is more common than aortic involvement.*E faecalis* caused most cases of endocarditis. Risk factors include urinary tract infection or instrumentation.Most cases of enterococcal bacteremia are not associated with endocarditis. Indeed, only about 1 out of 50 cases of enterococcal bacteremia results in endocarditis[91]

W. G. Jones, P. S. Barie et al have done a retrospective study to examine the incidence and clinical significance of enterococcal bacteremia in burns patients with enterococcal burn-wound infections. Mortality was significantly greater for bacteremic patients than for patients with enterococcal wound infection alone or for burns patients without enterococcal infections[99].

Shrikiran Hebbar et al have reported their experience with a case of enterococcal meningitis in a healthy 7 month old infant who did not present with any predisposing factors.They have discussed the role of enterococci in the pathogenesis of meningitis.According to them, Enterococci are clearly unusual etiological agents of bacterial meningitis[86].

Chiara Iaria et al have reported a case of enterococcal meningitis due to Enterococcus casseliflavus.They have reviewed that Enterococcal meningitis accounts for only 0.3% to 4% of cases of bacterial meningitis which is nevertheless associated with a high mortality rate. It has been described most frequently in patients with neurosurgical conditions[12].

Sherwood L Gorbach et al has stated that most cases of enterococcal meningitis occur in patients with anatomic defects of the central nervous system, prior neurosurgery, or head trauma. Meningitis is a rare complication of high-grade bacteremia in patients with enterococcal endocarditis. Meningitis also occasionally complicates enterococcal bacteremia in patients with severe immunodeficiencies including acquired immunodeficiency syndrome and acute leukemias. It is also seen in neonatal sepsis[85].

Stroud, L., J.Edwards et al has said that respiratory tract infections due to enterococci are exceedingly unusual. Although well-documented cases of enterococcal pneumonia and even lung abscess exist, they usually occur in patients with severe and debilitating diseases. Broad-spectrum antimicrobial therapy, especially with cephalosporins, coupled with enteric feeding in severely debilitated patients has been the setting in which some of the rare cases of enterococcal pneumonia have been described[90].

James F. Morris et al have stated that Enterococci are rarely implicated in the causation of pneumonia or lung abscess. They have reported two patients who have developed large lung abscess cavities from enterococcal pneumonia. Combination of penicillin and aminoglycoside antimicrobial therapy and drainage with a transthoracic tube resulted in complete cavity closure and healing in these patients [30].

Mehrdad Behnia et al have said that the most common organisms isolated from empyema are *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Hemophilus influenzae*, *Escherichia coli*, *Klebsiella pneumoniae*, and species of *Bacteroides*. Enterococcus is rarely present in chest infections.They have reported Enterococcus faecalis causing empyema in a case of Liver disease[56].

Stroud, L., J.Edwards et al states that Enterococci have clearly been documented to cause neonatal sepsis characterized by fever, lethargy, and respiratory difficulty accompanied by bacteremia or meningitis, or both. Although early-onset bacteremia in otherwise normal neonates is characteristic of this disease, several nosocomial outbreaks of bacteremia or meningitis, or both, due to *E.faecium* or *E.fecalis* have been described in premature or low-birth-weight neonates who had nasogastric tubes and intravascular devices. In general, neonates with enterococcal sepsis have responded well to appropriate antimicrobial therapy[90].

Patrick & Murray et al in the Text book of Manual of Clinical Microbiology have described the laboratory isolation of enterococci. Trypticase soy – 5% sheep blood agar, brain – heart infusion – 5% sheep blood agar or any blood agar base containing 5% animal blood supports the growth of Enterococci. Some strains of E.faecalis are β - hemolytic on agar base containing rabbit or horse blood but non haemolytic in the same base media containing sheep blood. If the sample is likely to contain gram negative bacteria, bile-esculin agar and azide, Pfizer selective Enterococcus medium are excellent primary isolation media[56]. The azide – inhibits the Gram – negative bacteria and the Enterococci appear as black colonies because of hydrolysis of aesulin[66].

Bailey & Scott's Text book of Diagnostic Microbiology, 11th edition describe the use of other media such as columbia –colistin – nalidixic agar (CAN) or phenyl ethyl alcohol agar (PEA) to isolate Enterococci. The advantage of CAN over PEA is that haemolytic reaction can be read from CAN. With increasing evidence of Vancomycin Resistant Enterococci, Enterococcosel broth, brain heart infusion broth containing vancomycin 6µg/ml., VRE selection medium (bile esculin azide agar containing vancomycin 6µg/ml) are useful selective media[9].

Topley & Wilson, Text book of Microbiology and Microbial Infections , states that the colonies on blood agar media are usually between

1-2 mm in diameter. Some cultures of E.faecalis may be β - hemolytic on agar containing rabbit, horse or human blood but non haemolytic on agar containing sheep blood. Some culture of Enterococcus durans are β haemolytic regardless of the type of blood used. All other species are either α or non haemolytic. Enterococcus casseliflavus, E.mundtii and E.sulfureus produce a yellow pigment on blood agar medium. The pigment is detected by using a white cotton swab to pick up the growth and examining the swab of yellow colour. In bile-esculin-azide medium, the colonies appear grey – white surrounded by a black halo[93].

M.Ford et al have used Cephalexin Aztreonam Agar for the selective isolation of Enterococcus faecium.E.faecium was differentiated from E.faecalis and E.durans by their ability to ferment arabinose on CAA[43].

Jawetz, Melnick and Adelberg in the Text book of Medical Microbiology have stated that resistance to the inhibitory and bactericidal activities of several commonly used antimicrobial agents is a remarkable characteristic of most of the enterococcal species. Antimicrobial resistance markers can be either intrinsic or acquired. Intrinsic (or) inherent resistance traits are present in all or most of the strains and hence appears to be chromosomally coded[31].

Y.A. Marothi et al has reviewed the enterococcal resistance. Antimicrobial resistance in enterococci is of two types: inherent/ intrinsic resistance and acquired resistance. Intrinsic resistance is species characteristics and thus present in all members of species and is chromosomally mediated. Enterococci exhibits intrinsic resistance to penicillinase susceptible penicillin (low level), penicillinase resistant penicillins, cephalosporins, lincosamides, nalidixic acid, low level of aminoglycoside and low level of clindamycin. Although most enterococci are susceptible to co-trimoxazole *in vitro* , this combination does not work *in vivo* , because enterococci are able to incorporate preformed folic acid which enables them to bypass the inhibition of folate synthesis produced by co-trimoxazole. On the other hand, acquired resistance results from either mutation in DNA or acquisition of new DNA. Examples of acquired resistance include resistance to penicillin by β -lactamases, HLAR, vancomycin, chloramphenicol, erythromycin, high level of clindamycin, tetracycline and fluroquinolone resistance[103].

R. Fontana et al in their publication have described two mechanisms which are responsible for resistance of enterococci to beta-lactam antibiotics: alterations of penicillin-binding proteins and production of a beta-lactamase. The latter has been found in a few clinical isolates of *Enterococcus faecalis*, whereas the former appears to account for resistance in most strains[71].

Strains expressing high level resistance to aminoglycosides have minimal inhibitory concentrations (MIC ≥ 2000 μg/ml) due to Enterococcal aminoglycoside modifying enzymes and cannot be detected by diffusion test with conventional discs[41]. To circumvent this problem, special test using high content gentamycin (120 μg) and streptomycin (300 μg) discs were developed to screen for their type of resistance as documented in strains of E.avium, E.casseliflavus, E.gallinarium, E.raffinosus and E.mundtti. It is transposon mediated[93].

Vandana A et al has reported concomitant HLPR and HLAR in 16% of enterococci.Low level vancomycin resistance was encountered by them in 3.3% enterococci[97].

S.Parvathi,B.Appala Raju et al have done a comparative evaluation of β-lactamase production in enterococci by Acidometric and clover leaf technique[81].

Topley & Wilson in the Text book of Microbiology and Microbial Infections has stated that strains producing β lactamase through plasmid mediated gene, occur sporadically. This can be detected by using chromogenic cephalorsporin assay[93].

Mackie & Mc Cartney in their Text book of Microbiology and Microbial Infections have said that β-lactamase production is reported in E.faecalis, E.faecium, E.gallinarum & E.raffinosus also. Gene encoding for the

Enterococcal β-lactamase is the same gene as found in Staphylococcus aureus. The gene is constitutively expressed in Enterococci and inducible in Staphylococci. Because Enterococci may produce small amounts of the enzyme, they may appear to be susceptible to penicillin and ampicillin by routine susceptibility test[46].

Patrick & Murray et al in the Text book of Manual of Clinical Microbiology has said that the β-lactamase can be detected using a high inoculum and the chromogenic cephalosporin test or by other methods. Infections due to β-lactamase producing Enterococci can be treated with combination of penicillin and β lactamase inhibitor or vancomycin and streptomycin when in vitro susceptibility has been demonstrated[66].

Rosemeire Cobo Zanella et al have reviewed the resistance of Vancomycin-resistant enterococci (VRE). Vancomycin-resistant enterococci were first reported in 1988 in Europe, and then they have emerged and disseminated as an important cause of nosocomial infections worldwide. Glycopeptide resistance in enterococci is associated with a variety of phenotypes and genotypes. Two principal phenotypes of resistance in VRE have been described and associated with nosocomial infections. In enterococci, the *van*A phenotype is characterized by high-level resistance to vancomycin and teicoplanin, whereas the VanB phenotype by vancomycin resistance and teicoplanin susceptibility[79].

Bell, J. M., Paton, J.C. et al have reviewed that genes encoding the *van*A- and *van*B-phenotype resistance are located on transposons Tn*1546* and Tn*1547*, respectively, or in closely related transferable genetic elements. A third type of vancomycin resistance, termed VanC, has been known for many years to be a natural (intrinsic) vancomycin resistance found in the motile enterococci *E. casseliflavus*, *E. gallinarum*, and *E. flavescens*[10].

Mandell, Douglas & Bennett et al in the Text book of Principles and Practice of Infections has stated that Enterococci show resistance to even oxazolidinones, a new antibiotic that shows excellent activity against Gram positive organisms as per the recent reports[48].

Johnson et al has stated that Vancomycin Resistant Enterococci (VRE)show susceptibility to quinopristin – dalfopristin a new antibiotic of streptogramin group. Daptomycin, a lipopeptide, a new bactericidal antibiotic approved by FDA in 2003 is highly active against most Gram positive cocci, including Vancomycin Resistant Enterococcus faecalis[33].

Enterococci often show susceptibility to Trimethoprim – sulfamethoxazole in vitro testing but these drugs are not effective in treating infection. This is because in vivo, Enterococci utilize exogenous folates, thus escape inhibition by the drugs[48].

Enterococci, being a leading cause of nosocomial infections and frequently exhibiting multiple antibiotic resistance, typing & subtyping the isolates is necessary as a means of assisting infection control and epidemiological studies. It includes bacteriocin typing, phage typing, bio-chemical reaction profiles, antimicrobial patterns, and serological characterisation[93].

Bacteriocins are proteins that prevent the growth of other bacteria. Among Enterococci, E.faecalis, E.faecium and E.hirae are known to produce bacteriocins. These are inhibitors to Listeria monocytogenes, Staphylococcus, Lactococcus sp. and other Enterococcus sp. Bacteriocin production is linked to conjugative plasmid in some cases. The haemolytic activity of E.faecalis is linked to bacteriocin activity and both these activities are associated with a conjugative plamid[93]. Haemolysin activity is associated with strain virulence[77]. Bacteriocins are used as food preservatives, used as a typing system for Enterococci.

The virulence of enterococci have been reviewed by **D. Morrison et al .** Cytolysin ,haemolysin, aggregative substances and adhesins are the most studied. The haemolysin is a cytotoxin that lyses human, horse and rabbit erythrocytes. Its production is coded by plasmid pAD1, which also confers bacteriocin production. Production of haemolysin was reported to be more common in strains of E.faecalis isolated from human infections than in strains isolated from faecal sources of healthy persons.Haemolysin and aggregative substance contributed to enhanced virulence in experimental endocarditis. These substances have an effect on colonisation, invasion, platelet aggregation or fibrin generation [14].

Enterococci are capable of transmitting genetic information by both plasmid and transposon exchange among themselves and with other genera. Plasmid exchange is through production of sex pheremones, this was observed in E.faecalis[77]. The genetic material on plasmids could be either drug resistance or virulence genes.

Enterococci are also capable of genetic exchange by conjugative transposons, which requires cell to cell contact. Transposons carry resistance determinants for antimicrobial agents such as tetracycline, erythromycin, gentamycin, kanamycin, and other aminoglycosides[77].

Mandell, Douglas & Bennett in the Text book of Principles and Practice of Infectious diseases, 6th edition have given a detailed description regarding the treatment of enterococcal infection.Treatment of Enterococcal infection is complicated . For treating urinary tract infections, peritonitis, and wound infections, that do not require any bactericidal treatment, ampicillin remains the antibiotic of choice. Vancomycin is the drug of choice for the patients who are allergic to penicillin or those with high level penicillin resistant organisms. 90 – 96% remain susceptible to nitrofurantoin, successfully used to treat urinary tract infection. Fosfomycin is also effective. Increasing resistance to ciprofloxacin and ofloxacin is reported in vitro but sparfloxacin, levofloxacin, gepafloxacin and trovofloxacin are more active. Now, Enterococci are exhibiting resistance to erythromycin and related macrolides. Tetracycline and chloramphenicol may exhibit in vitro activities against these organisms and clinical failures of chloramphenicol are documented.Combination therapy is optimal for Enterococcal endocarditis and for meningitis as well. Combination of cell wall active agents (usually penicillin, ampicillin or vancomycin) with aminoglycosides (usually streptomycin or gentamycin) have been the standard for treatment of Enterococcal endocarditis since the first demonstration of penicillin – streptomycin synergy in 1947. Several antimicrobial agents show some promise for the therapy of infection due to multiple drug resistant Enterococci especially Vancomycin Resistant Enterococci. They include the streptogramin combination quinopristin – dalfopristin, an oxazolidinone linezolid, the everninomicin ziracin, a new semisynthetic glycopeptide, LY 333328 and ramoplanin, a novel glycolipodesipeptide[48].

Richard L. et al has said that β lactamase producing strains remain susceptible to vancomycin (and teicoplanin) and to combinations of β lactam and β lactamase inhibitors such as ampicillin – sulbactam and amoxicillin – clavunlanate[77].

Centers for Diseases Control and Prevention, have recommended methods for preventing the spread of vancomycin resistance.The appearance and spread of multiple resistant strains of VRE has prompted the adoption of new strategies to prevent infection with these Enterococci in hospitalised patients.

The recommendations include:-

- Guidelines for prudent use of vancomycin
- Education of hospital staff
- Early detection and prompt reporting of new VRE cases
- Implementation of barrier precautions to interrupt nosocomial transmission, especially that mediated by the hands of health care workers[11].

Richard L. Gurret et al in the Journal of Tropical infectious diseases has said that vancomycin use in the hospital may foster the spread of resistance if used inappropriately; hence the HICPAC recommendations promote better usage.

Education of hospital staff is essential for any VRE control effect. The importance of person to person spread requires emphasis as does the potential role of contaminated surfaces. Fortunately, VRE strains are susceptible to most environmental disinfectants, but they may be used in accordance with the manufacturer's recommended time of exposure[77].

MATERIALS AND METHODS:

This Prospective study was conducted at Government Rajaji Hospital, attached to Madurai Medical College after getting ethical clearance from the Human Ethical Committee, GRH, headed by the Dean. The period of study was 4 months from November 2006 to February 2007 . The study population consisted of 200 patients admitted in different wards viz Paediatrics ,General Surgery, Urology and Medicine at Government Rajaji Hospital,Madurai. Various specimens like Blood ,Urine, Pus Swab and CSF were collected from the patients depending on the clinical symptoms.

INCLUSION CRITERIA

- Septicemic and PUO cases in Paediatric ward .
- Wound infection following burns in Surgery ward.
- Post operative wound infection in Surgical Ward.
- Intraabdominal abscesses in Surgical Ward.
- Cases with Urinary tract infection in Urology Ward
- Meningitis and Endocarditis cases in Medicine ward.

EXCLUSION CRITERIA

- All other cases from Pediatric ward.
- All other wound infections from Plastic Surgery ward.
- All other abscesses in Surgical ward
- All other cases from Medical Ward
- Other infections in Urology Ward.

General examination was done on each patient. Basic investigations like Hb, TC, DC, ESR, and Blood sugar were done on each patient and recorded. Antibiotics administered to these patients with the dosages and the period of administration were noted.

COLLECTION OF SPECIMENS:-

Blood was collected with strict aseptic precautions. A tourniquet was placed above the venepuncture site. Using 70% alcohol, the venepuncture site was cleaned in a circle approximately 5 cm in diameter rubbing vigorously. The alcohol was then allowed to air dry. Next ,10% povidone Iodine was applied and the skin was swabbed concentrically from the centre of the venepuncture site outwards. The iodine was allowed to act on the skin for atleast 1 minute. Precautions were taken not to repalpate the vein. The venepuncture site was then punctured. In adults ,5ml of blood was drawn aseptically and the blood was transferred to the blood culture bottle with 50ml of BHI broth swabbing the cap with 70% alcohol .Blood was then inoculated into the bottle through the hole in the cap. The caps were then secured tightly. In children, 1-2ml of blood was collected similiarly in 20ml of sterile BHI broth in blood culture bottles. If the infant had an existing IV line, blood was drawn below the existing line. In Endocarditis, three samples were collected one hour apart.

Pus samples were collected in syringes or swabs. The site of lesion of the wound was decontaminated with surgical soap and 70% Isopropyl alcohol. The wound was then washed with sterile saline and allowed to dry. The discharge was then collected in a sterile syringe. If the discharge was minimal, sterile swabs were used. The wound margins were separated and swabs were taken from the depth of the wound. Care was taken not to involve the skin margins. Two swabs were taken, one for direct microscopic examination and the other for culture.

Urine samples were collected by sampling the mid stream flow by the clean catch technique. Appropriate instructions were given to the patients and the specimen was collected in a wide mouthed container.

CSF was collected in cases with meningitis. Spinal tap was done with the assistance of an experienced physician. The patient was asked to lie on his or her side. The area overlying the lumbar spine was disinfected and

CSF was collected by introducing a spinal needle between L3 and L4 lumbar spaces.

TRANSPORTATION OF SPECIMEN:

After collection, the samples were checked for proper labelling like Patient's name, age sex, IP/OP Number. All the clinical samples collected were transported to the Microbiology laboratory within 1 hr of collection.

PROCESSING OF SPECIMENS:

In the Microbiology Laboratory, the specimens were checked for any leakage (or) damage. The macroscopic appearance of the specimens such as colour, turbidity and odour were noted. Blood culture bottles were incubated at 37ºC for 48 hrs. Direct microscopic examination was done for Pus and CSF samples. The smears prepared from the above samples were stained with Gram's stain and observed under the microscope. The specimens were plated on Nutrient Agar, MacConkey and Blood Agar plates and incubated at 37ºC overnight. The colony morphology on the plates were then noted. On Nutrient Agar plate, entercoccus colony was small 0.5 – 1 mm transparent, low convex, discrete with glossy surface. On MacConkey Agar, they produced 0.5-1mm sized, magenta coloured lactose fermenting colonies. Blood agar plate showed α, β, or γ hemolysis. The colonies with the above characteristics were presumed to be enterococcus. Further confirmation of the isolate as Enterococcus was done by Gram's stain, Catalase test, Bile Esculin hydrolysis, Heat resistance and Salt tolerance test. In Grams stain, Enterococci appeared as Gram positive cocci arranged in pairs and short chains with a characteristic leaf shape. Further the organisms were found to be catalase negative . The culture was then inoculated onto Bile esculin agar plates and incubated at 37^0C for 48 hrs. Enterococci and group D Streptococci produced blackening of the agar plates.. The organisms were further subjected to heat test. For this test, 2 or 3 of the isolated colonies were inoculated into glucose broth and incubated at 37^o C overnight. The growth was judged with the turbidity, sub cultured from the broth onto one half on a nutrient agar plate, then the broth was placed in a water bath set at 60^o C for 30 minutes. Sub culture was done from the broth on the other half of the nutrient agar medium. After incubation at 37^o C for 24 hrs, growth was observed on both halves of the plate, which indicates the heat resistant property of Enterococci.Next salt tolerance test was done. This is based on the ability of the Enterococci to grow in the presence of 6.5% NaCl incorporated into broth/agar while other Group D Streptococci are negative for this test.

The enterococcus isolates were further speciated into E. faecalis, E. faecium and E. durans depending upon the fermentation of sugars, motility, arginine decarboxylation, pigmentation, reduction of tellurite and using selective media. The common sugars used were Arabinose, Raffinose, Sucrose, Sorbitol and Mannitol. For sugar fermentation test, Todd-Hewitt broth with the 1% sugar and bromothymol blue as indicator was used. Each tube was inoculated with 2 drops of an 18-24 hrs brain-heart infusion broth culture and incubated at 37ºC for 24-48 hrs and observed for colour change from blue to yellow.Pyruvate fermentation was tested by inoculating fresh culture into pyruvate broth and incubated at 37ºC for 24-48 hours. Change in color from blue to yellow showed fermentation of pyruvate.

All the three species did not ferment Raffinose . Arabinose was fermented by E. faecium. Pyruvate was fermented by only E. faecalis. The above three species were found to be non motile by hanging drop and by Mannitol motility medium.

All the above three species of Enterococcus deaminated the aminoacid Arginine to ammonia resuting in alkalinization of the medium thus changing the colour from yellow to purple.

Pigmentation produced was tested by touching the colony grown on Trypticase Soy Agar with a Dacron swab and noting whether yellow pigment was produced or not. All the three species, E. faecalis, E. faecium and E. durans did not produce any pigmentation. The isolates were then tested for reduction of tellutrite.The medium used was human blood agar plate incorporated with 0.04% pottasium tellurite. The isolated colonies were streaked on the plate, incubated at 37º C for 24-48 hours. Enterococcus faecalis isolates were identified by the brownish black colored colonies.The other two species did not reduce potassium tellurite into metallic tellurium.

The identification of the three species were done as follows:

Species	Motility	Arabinose	Raffinose	Sucrose	Sorbitol	Mannitol	Pyruvate	Arginnine	Pot.tellurite
E. faecalis	-	-	-	+-	+ -	+	+	+	+
E. faecium	-	+	-	v	-	+	-	+	-
E. durans	-	-	-	-	-	-	-	+	-

The selective media used was Hicrome Enterococcus agar base with Arabinose, Chromogenic substrate, peptone and corn starch as main ingredients, phenol red as indicator with added E.faecium Selective Supplement. E. faecium was identified by the conversion of the colonies into green colour with the surrounding medium changing yellow due to arabinose fermentation. E. faecalis produced blue colour colonies.E. durans did not show any change.

The different species of enterococci were further subjected to antimicrobial susceptibility testing by the Kirby Bauer Method . The antibiotics used were Erythromycin, Ampicillin, High level Gentamycin, Ciprofloxacin, Doxycycline,Nitrofurantoin,Ceftriaxone ,Cefataxime and Vancomycin. A few colonies of the isolates were inoculated into peptone water, which was standardized to a density equivalent to 0.5 McFarland opacity. The inoculum was then spread across the surface of Mueller Hinton agar plate of 9 cm. size to give a confluent growth.Filter paper discs of 6 mm size containing the specific concentration of the antibiotics were placed on the agar surface 24 mm apart. Six discs were kept on the surface of the plate at a time. The plates were incubated over night at 37ºC for 24-48 hrs. After incubation the diameter of the zone of growth inhibition was measured by a graduated ruler and the sensitivity and resistant pattern were identified as follows:

S. No	Antibiotic	Disc content	Zone of inhibition (mm)	
			Sensitive	Resistant
1	Ampicillin	10 µg	≥ 17	≤ 16
2	Gentamycin	120 µg	≥ 10	6
3	Ciprofloxacin	5 µg	21	15
4	Nitrofurantoin	300 µg	≥ 17	≤ 14
5	Doxcycline	30 µg	16	12
6	Erythromycin	15 µg	≥ 18	≤ 13
7	Ceftriaxone	30 µg	> 21	≤ 13
8	Cefataxime	30 µg	> 23	≤ 14
9	Vancomycin	30 µg	≥ 17	≤ 14

The principle, procedure and interpretation of the tests are given in the annexure-1.

RESULTS

In this study, a total of 200 samples, 50 samples each from Paediatrics, Surgery , Medicine and Urology ward at Govt. Rajaji Hospital were collected and analysed for the Species of enterococcus commonly involved in the infections of this hospital , the age group and sex commonly affected by this species of enterococcus, the common infection occurring in the patients affected by this species and the most common factor responsible for the occurrence of this infection in this hospital.

The 200 samples collected were analysed age and sex wise. Out of the 30 samples collected between the age group 0-1month ,19(63.3%) were from males and 11 (36.6%)were from females. Out of the 12 samples collected between the age group 1 month-12months , 8(66.6%) were males and 4(33.3%) were females. Out of 8 samples from the age group 1-12 years, 4 (50%) samples each were collected from males and females. Out of 50 samples collected between the age group 13-33 yrs, 16 (32%) were from males and 34 (68%) were from females. Out of 80 samples from the age group 34-54 yrs, 38 (47.5%) were from males and 42 (52.5%) were from females. Above the age group of 54 yrs, 20 samples were collected, out of which 12 (60%) were from males and 8 (40%) were from females. . **It was found that in males, more number of samples were collected in the age group 1 month to 12 months and in females between the age group 13- 33 years**.. The age and sex distribution of samples are given in **Table 1**

TABLE 1: AGEWISE AND SEXWISE DISTRIBUTION OF SAMPLE

S.No.	Patient Age	Number	Sex	Number	%
1.	0-1 month	30	Male	19	63.3%
			Female	11	36.6%

2.	**1 month – 12 months**	12	**Male**	**8**	**66.6%**
			Female	4	33.3%
3.	1-12 yrs	8	Male	4	50%
			Female	4	50%
4.	13-33 yrs	50	Males	16	32%
			Female	**34**	**68%**
5.	34-54 yrs	80	Male	38	47.5%
			Female	42	52.5%
6.	>54 yrs	20	Male	12	60%
			Female	8	40%
Total		200		200	

Among the 200 samples, 50 samples were collected from the Paediatric ward. Out of the 50 samples collected, 24 (48%) were urine, 24(48%) were blood, 1 each from CSF and pus (2% each). Among the 50 samples collected from the General Surgery, 8(16%) were Urine, 12(24%) were blood, 17(34%) were Pus, 13(26%) were Swab and there was no CSF sample. Among the 50 samples collected from the Medical ward, 19(38%) were Urine, 26(52%) were blood, 4(8%) were CSF and 1(2%) was from

Pus .From Urology ward, a total of 50 samples were collected, out of which 33(66%) were from Urine, 17(34%) were Blood and there was no pus/ swab/ CSF.. The distribution of samples ward wise and specimen wise is given in **Table no.2**

TABLE 2

WARDWISE AND SPECIMEN WISE DISTRIBUTION OF SAMPLE

S.No	Ward	Total no of Specimens	Specimen				
			Urine	Blood	CSF	PUS	Pus/Wound Swab
1	Pediatric	50	24 (48%)	24(48%)	1(2%)	1(2%)	-
2	General Surgery	50	8(16%)	12(24%)	-	17(34%)	13(26%)

3	Medicine	50	19(38%)	26(52%)	4(8%)	1(2%)	-
4	Urology	50	33(66%)	17(34%)	-	-	-
Total		200	84(42%)	79(39.5%)	5(2.5%)	19(9.5%)	13(6.5%)

It was found that urine and blood were the common specimens collected in pediatric ward, pus and swab in general surgery ward, blood in medical ward and urine in urology ward.

All the 200 samples were subjected to gram staining. It was found that 71 out of 200 (35.5%) were gram positive cocci. The samples were further analysed ward wise and specimen wise as per Gram reaction. The percentage of Gram positive cocci isolated from Pediatrics, General surgery, Medicine and Urology wards was 52% (26 out of 50), 28% (14 out of 50), 26% (13 out of 50) and 36% (18 out of 50) respectively. **Thus wardwise distribution showed that more number of Gram positive cocci were isolated from the Pediatric ward**. The percentage of Gram positive cocci isolated from Urine, Blood, Pus, Swab and CSF was 23.8% (20 out of 84), 54.4% (43 out of 79), 21% (4 out of 19), 23% (3 out of 13) and 20% (1 out of 5) respectively. **Thus specimenwise distribution showed that more Gram positive cocci were isolated from blood.** The wardwise and specimenwise distribution of Grampositive cocci are given in **Table no.3 and 4**

TABLE 3

WARDWISE DISTRIBUTION OF GRAM POSITIVE COCCI

S.No	Ward	Total no of sample collected	Gram Positive Cocci	%
1.	**Pediatric Ward**	**50**	**26**	**52%**
2.	General surgery Ward	50	14	28%
3.	Medicine Ward	50	13	26%
4.	Urology Ward	50	18	36%
	TOTAL	**200**	**71**	

TABLE 4

SPECIMENWISE DISTRIBUTION OF GRAM POSITIVE COCCI

S.No	Specimen	Total No of specimen collected	Gram positive cocci	%
1	URINE	84	20	23.8%
2	**BLOOD**	**79**	**43**	**54.4%**
3	PUS	19	4	21%
4	SWAB	13	3	23%
5	CSF	5	1	20%

	Total	200	71	

All the 71 Gram positive cocci were processed for the presence of enterococcus and by confirmation using the various tests and it was found that 36 out of 71 (50.7%) were Enterococci. The other Gram positive cocci isolated were Coagulase Negative Staphylococci (CONS) 24 (33.8%) and Staphylococcus aureus 11 (15.4%). Sexwise analysis of the 36 enterococcal isolates showed that in Pediatric ward,7 out of 12 isolates were from males (58.3%) and 5 were from females (41.6%) In general surgery,among the 8 enterococcus isolates, 5 (62.5%) were males and 3 (37.5%) were females. In medical ward, out of 6 isolates 4 (66.6%) were males and 2 (33.3%) were females. In the urology ward,out of 10 enterococcus isolates, 4 were from males(40%) and 6 were from females(60%).**Analysis of sexwise distribution of enterococcus isolates showed that males predominated in all the wards except urology where the females predominated.** The isolates were analysed wardwise and it was found that 12(33.3%) were from pediatric ward, 8 (22.2%),were from Surgery ward,6(16.6%) were from Medical ward and 10 (27.7%) were from Urology ward. **Thus maximum number of isolates of enterococcus were from Pediatric ward**. Wardwise and sexwise distribution of enterococcus isolates is given in **Table no.5.**

TABLE 5

WARDWISE AND SEXWISE DISTRIBUTION OF ENTEROCOCCUS

S.No.	Ward	Total Number	Sex	Number	%
1.	Pediatric	12	Males	7	58.3%
			Females	5	41.6%
2.	General Surgery	8	Males	5	62.5%
			Females	3	37.5%
3.	Medicine	6	Males	4	66.6%
			Females	2	33.3%
4.	Urology	10	Males	4	40%
			Females	6	60%
		36		36	

The 36 enterococal isolates were further analysed agewise. It was noted that 10(27.7%) out of 36 were in the age group 0-1 month , 3(8.3%) were in the age group of 1 month – 12 months , 2 (5.5%) were between 1 – 12 yrs, 7(19.4%) were in the age group of 13 – 33 yrs, 8(22.2%) were in the age group 34 – 54 yrs and 6(16.6%) were in the age group more than 54. **It was noted that maximum number of isolates of enterococcus were between the age group 0-1 month**. The agewise distribution of enterococcus is given in **Table 6**.

TABLE 6

AGE WISE DISTRIBUTION OF ENTEROCOCCUS

PATIENT'S AGE IN YEARS	NUMBER	%

0-1 month	**10**	**27.7%**
1 month – 12 months	3	8.3%
1 – 12 yrs	2	5.5%
13 - 33yrs	7	19.4%
34 – 54 yrs	8	22.2%
> 54 yrs	6	16.6%

The enterococcus species were analysed specimenwise and it was found that out of the 36 Enterococcal isolates, 14 (38.8%) were from urine, 20 (55.5%) were from blood, 1 (2.7%) was from pus, 1 (2.7%) was from Swab and no isolate from CSF. **Thus maximum number of enterococci were isolated from blood samples.** Specimenwise distribution of enterococcus isolates is given in **Table 7.**

TABLE 7

DISTRIBUTION OF ENTEROCOCCI SPECIMEN WISE

SPECIMEN	Number isolated	PERCENTAGE
URINE	14	38.8%
BLOOD	**20**	**55.5%**

PUS	1	2.7%
SWAB	1	2.7%
CSF	-	-
Total	36	50.7%

Inpatient and outpatient analysis of Enterococcal isolates showed that 28 out of 36(77.7%) were from Inpatients and 8 out of 36(22.2%) were from outpatients thus showing inpatient predominance .The incidence of inpatient and out patient enterococcus isolates is given **in Table no.8**

Table – 8

INCIDENCE OF ENTEROCOCCUS - INPATIENT, OUTPATIENT

IP/OP	NO. OF ISOLATES	PERCENTAGE
Inpatient	28	77.7%
Outpatient	8	22.2%

Enterococcus were speciated into E.faecalis, E.faecium and E.durans according to the biochemical reactions and it was found that out of the 36 Enterococcus isolates, 15(41.6%) were E.faecalis,18 (50%) were E.faecium, and 3 (8.3%) was E.durans. **Thus the most common species of enterococcus isolated was E. faecium.** The species wise distribution of Enterococcus is given in **Table no:9**

TABLE 9

SPECIES WISE DISTRIBUTION OF ENTEROCOCCUS

SPECIES	ISOLATES IN NUMBER	PERCENTAGE
E.faecalis	15	41.6%
E.faecium	18	50%
E.durans	3	8.3%

The three species were analysed age wise and it was found that in the age group 0-1 month 9(90%) were E.faecium, 1(10%) was E.durans. In the age group 1 month – 12 months 1(33.3%) was E.faecalis and 2(66.6%) were E.faecium. In the age group 1-12 years 1(50%) was E.faecalis and 1(50%) was E.faecium. In the age group 13-33 years 5(71.4%) were E.faecalis, 1(14.2%) was E.faecium and 1(14.2%) was E.durans. In the age group 34-54 years 4(50%) were E.faecalis, 3(37.5%) were E.faecium and 1(12.5%) was E.durans. In the age group more than 54years 4(66.6%) were E.faecalis and 2(33.3%) were E.faecium. **It was found that 50% were E. faecium isolates and it was commonly found in the age group between 0-1 month.** Agewise distribution of enterococcus species is given in **Table no.10.**

TABLE - 10

AGEWISE DISTRIBUTION OF ENTEROCOCCUS SPECIES

S.No	Age	Total no. of enterococcus isolates	E.faecalis		E.faecium		E.durans	
			Total Number	%	Total Number	%	Total Number	%
1	**0-1 month**	10	-	-	9	**90%**	1	10%
2	1– 1 months	3	1	33.3%	2	66.6%	-	-
3	1yr – 12 yrs	2	1	50%	1	50%	-	-
4	13- 33 yrs	7	5	71.4%	1	14.2%	1	14.2%
5	34 – 54 yrs	8	4	50%	3	37.5%	1	12.5%
6	> 54 yrs	6	4	66.6%	2	33.3%	-	-
	Total	36	15	41.6%	18	**50%**	3	8.3%

The sexwise distribution shows that in pediatric ward out of 7 males 1(14.2%) was E.faecalis, 5(71.4%) were E.faecium and 1(14.2%) was E.durans. In Surgery ward out 4 males 3(75%) were E.faecalis, 1(25%) was E.faecium and no E.durans. In Medicine ward out of 4 males 2(50%) was E.faecalis, 1(25%) was E.faecium and 1(25%) was E.durans. In Urology ward out of 4 males 1(25%) was E.faecalis, 3(75%) were E.faecium and no E.durans. In pediatric ward out 5 females 1(20%) was E.faecalis, 4(80%) were E.faecium and no E.durans. In Surgery ward out of 4 females 2(50%) were E.faeclis, 2(50%) were E.faecium and 1(25%) was E.durans. In Medicine ward out of 2 females 1(50%) each were E.faeclis and E.faecium. In Urology ward out of 6 females 5(83.3%) were E.faecalis and 1(16.6%) was E.faecium. Sexwise distribution of enterococcus species is given in **Table no.11.**

TABLE 11

SEXWISE DISTRIBUTION OF ENTEROCOCCUS SPECIES

Ward	Sex	Total entero coccus	E.faecalis		E.faecium		E.durans	
			Total Number	%	Total Number	%	Total Number	%
Pediatric	**Male**	7	1	14.2%	5	71.4%	**1**	14.2%
	Female	5	1	20%	4	80%	**-**	
Surgery	**Male**	4	3	75%	1	25%		
	Female	4	2	50%	2	50%	1	25%
Medicine	**Male**	**4**	**2**	50%	**1**	25%	**1**	25%
	Female	**2**	**1**	50%	**1**	50%	**-**	
Urology	**Male**	**4**	**1**	25%	**3**	75%	**-**	
	Female	**6**	**5**	83.3%	**1**	16.6%	**-**	

The three different species were analysed wardwise and it was found that in Paediatric ward, out of 12 isolates 2(1.8%) were E. faecalis, 9(75%) were E. faecium, 1(2.7%) was E. durans. In Surgery out of 8 cases 4(50%) were E.faecalis, 3(37.5%) were E.faecium and 1 (12.5%) was E.durans. In Medicine, out of 6 isolates 3(50%) were E.fecalis, 2(33.3%) were E.faecium and 1(16.6%) was E.durans. In Urology, out of 10 isolates 6(60%) were E.faecalis and 4(40%) were E.faecium. **It was noted that E. faecium was the commonest species found in the pediatric ward.** Wardwise distribution of enterococcus species is given in **Table no.12.**

TABLE 12

WARDWISE DISTRIBUTION OF ENTEROCOCCUS SPECIES

S.No	Ward	E.faecalis		E.faecium		E.durans	
		Total Number	%	Total Number	%	Total Number	%
1	Pediatric (12)	2	1.8%	9	75%	1	2.7%
2	General Surgery (8)	4	50%	3	37.5%	1	12.5%
3	Medicine (6)	3	50%	2	33.3%	1	16.6%

4	Urology (10)	6	60%	4	40%	-	-

The three species of Enterococci were further analysed specimenwise and it was found that in urine 10(27.7%) out of 36 were E. faecalis, 2(5.5%) out of 36 were E. faecium, 2(5.5%) out of 36 was E. durans. In blood, 4(11.1%) out of 36 was E. faecalis, 14(38.8%) out of 36 were E. faecium and 1(2.7%) was E. durans. In pus, 2(5.5%) was E. faecium and in swab 1(2.7%) was E. faecalis **Thus it was found that E. faecium was isolated more from blood and E.faecalis more in urine.** Specimenwise distribution of the three species of Enterococci is given in **Table no. 13**

TABLE 13

SPECIMENWISE DISTRIBUTION OF ENTEROCOCCUS SPECIES

SOURCE	E.faecalis	E.faecium	E.durans
URINE	10 / 36 (27.7%)	2/ 36 (5.5%)	2/ 36 (5.5%)

BLOOD	4 /36 (11.1%)	**14 /36 (38.8%)**	1/36 (2.7%)
PUS	-	2 /36 (5.5%)	-
SWAB	1 /36 (2.7%)	-	
Total	15/36 (41.6%)	**18/36 (50%)**	3/36 (8.3%)

The susceptibility and resistance patterns of Enterococci were studied. Out of 18 E.faecium isolates, 17 (94.4%) were resistant to Ampicillin, 12 (66.6%) to Gentamycin, 13 (72.2%) to Ciprofloxacin, 11 (61.1%) to Doxycycline, 13 (12.2%) to Nitrofurantion, 1 (5.5%) to Vancomycin, 18 (100%) to both Ceftaxime and ceftrioxone, 12 (66.6%) to Erythromycin.

Out of 15 E.faecalis, 13(86.6%) were resistant Ampicillin**,** 6(40%) to Gentamycin, 4(26.6%) to Ciprofloxacin, 10(66.6%) to Doxycycline, 9(60%) to Nitrofurantion, 7 (46.6%) to Erythromycin. All 15 isolates were resistant to Ceftriaxone and Ceftaxime and all were sensitive to Vancomycin.

Out of 3 E.durans, 100% resistance were seen with Ciprofloxacin, Cefataxime and Ceftriaxone. All isolates were sensitive to Vancomycin and Gentamycin. Out of the 3 isolates 2(66.6%) were resisitant to Doxycycline, 2(66.6%) to Nitrofurantion , 1(33.3%) to Erythromycin,1(33.3%) to Ampicillin.

Thus it was found that among all 36 isolates ,100% resistance was to Ceftaxime and Ceftriaxone , 50 % resistance to Gentamycin, 88.5% resistance to Ampicillin and 2.7 % resistance to Vancomycin. **It was noted that among the species, E.faecium showed more resistance than other species.** The Antibiotic Sensitivity and Resistance pattern is given in **Table 14 and Table 15.**

TABLE 14

ANTIBIOTIC SENSITIVITY PATTERN IN ENTEROCOCCI SPECIES WISE

Organism	Total No of Isolates	Ampicillin	Gentamycin	Ciprofloxacin	Doxycycline	Nitrofurantoin	Vancomycin	Ceftriaxone	Ceftaxime	Erythromycin
E.faecium	18	1 (5.5%)	6 (33.3%)	5 (27.7%)	7 (38.8%)	5 (27.7%)	17 (94.4%)	0 (100%)	0 (100%)	6 (33.3%)
E.faecalis	15	2 (13.3%)	9 (60%)	9 (60%)	5 (33.3%)	6 (40%)	15 (100%)	0 (0%)	0 (0%)	8 (53.3%)
E.durans	3	2 (66.6%)	3 (100%)	0 (0%)	1 (33.3%)	1 (33.3%)	3 (100%)	0 (0%)	0 (0%)	2 (66.6%)
Total no. of isolates	36	5 (13.8%)	18 (50%)	14 (38.4%)	13 (36.1%)	12 (33.3%)	34 (94.4%)	0 (0%)	0 (0%)	16 (44.4%)

TABLE 15

ANTIBIOTIC RESISTANCE PATTERN IN ENTEROCOCCI -SPECIES WISE

Organism	Total No of Isolates	Ampicillin	Gentamycin	Ciprofloxacin	Doxycycline	Nitrofurantoin	Vancomycin	Ceftriaxone	Ceftaxime	Erythromycin
E.faecium	**18**	**17 (94.4%)**	**12 (66.6%)**	**13 (72.2%)**	**11 (61.1%)**	**13 (72.2%)**	**1 (5.5%)**	**18 (100%)**	**18 (100%)**	**12 (66.6%)**
E.faecalis	**15**	**13 (86.6%)**	**6 (40%)**	**4 (26.6%)**	**10 (66.6%)**	**9 (60%)**	**0 (0%)**	**15 (100%)**	**15 (100%)**	**7 (46.6%)**
E.durans	**3**	**1 (33.3%)**	**0 (0%)**	**3 (100%)**	**2 (66.6%)**	**2 (66.6%)**	**0 (0%)**	**3 (100%)**	**3 (100%)**	**1 (33.3%)**
Total no. of isolates	**36**	**31 (88.5%)**	**18 (50%)**	**20 (55.5%)**	**23 (63.8%)**	**24 (66.6%)**	**1 (2.7%)**	**36 (100%)**	**36 (100%)**	**20 (55.5%)**

DISCUSSION

A total of 200 clinical samples from varied infections of Pediatric, General Surgery, Medicine and Urology wards of Govt .Rajaji Hospital, Madurai were collected and analysed to find the species of enterococcus commonly isolated from Urine, Blood, CSF, Pus and wound Swab . They were further analysed agewise, sexwise and specimenwise to know the common age group,sex and the common infection involved by this species of enterococcus during a period of 4 months from November 2006 to February 2007.

In the present study, among the 200 samples collected it was found that 42% of samples collected were from urine and 39.5% samples collected were from blood. This is in accordance with the study by Steven Gordan et al who had shown that 57% of their samples were from urine[88] and Patrick Murray etal who had also shown that 36% of their samples were from blood[66]. As the most common sites for isolation of enterococcus are urinary tract and blood stream, more number of samples collected from these two infections are justified.

In this study 52% Gram postive cocci were isolated from Pediatric ward in Blood samples. Similar study by Wisplinghoff et al showed that 65% of Gram positive cocci were associated with Pediatric Blood stream infection[101]. L.F. Nimri et al also showed that 53.3% Gram positive cocci were in blood samples[37]. These two studies are in accordance with our study. The more incidence of Gram positive organisims isolated from the pediatric ward may be attributed to the extremes of age or due to invasive procedures like intravenous infusion or may be due to inappropriate antibiotic usage. The common occurrence of gram positive cocci in blood may be attributed to the entry of these organisims through the intravenous route from other sources like urine, motion etc. because most of these children had the infusion line in their lower extremeties.

In this study it was observed that 50.7% of Gram postive cocci isolated from various infections were enterococcus species. This is in accordance with the study of Louis B. Rice etal who also had shown that Enterococcus infection was responsible for more than 40% in their study on various infections[41]. The higher incidence of Enterococcus among Gram positive cocci may be due to the properties involved in the adherance to host tissue which are considered as important virulent factors for establishing infection by Enterococci. Also enterococci are intrinsically resistant to a wide range of antibiotic which notably include β - lactams and Aminoglycosides frequently used to treat infections with Gram positive organisms. Also they have ability to acquire resistance to antimicrobial agents through plasmids and transposons and chromosomal exchange or mutation.

Age wise distribution of enterococcus in this study showed that 33.3% of them were from the paediatric ward and 27.7% of the enterococci isolated in pediatric ward were between the age group **0-1 month** .

This is in accordance with the study by Al Otaibi et al who had reported 30% of Enterococcal bacteremia in **neonates**[5]. The occurrence of enterococcal bacteremia in neonates is obvious because of their poor development of immune system and emergence of virulent antimicrobial resistant enterococci in pediatric wards due to the constant wetting of beds by neonates and irrelevant usage of antibiotics in these wards. In this study, in the paediatric ward, 86.3% males showed enterococcus whereas only 13.7 % females showed enterococci. Similar study by A.S.M. Nawshad Uddin Ahmed in their analysis of cases had reported that 63% of males were with neonatal enterococcal septicemia[3]. This might be due to the better natural immunity shown by female children or by hormonal protection rendered to the female children.

The sexwise distribution of Enterococcal isolates showed that 55.5% were males and 45% were females. In all wards except Urology ,more than 50% isolates were from males and in Urology ward more than 50% isolates were from females. Vittal Prakash et al in their study also demonstrated that 56.5 % were males and 43.5% were females[98]. The increased incidence of enterococcal isolation among females in Urology ward may be attributed to the more number of Urine samples collected in ward and enterococcus is a known urinary pathogen. It is more common in females mainly due to the anatomical built of the female urethra. The less incidence in males may be due to the drier environment surrounding the meatus and antibacterial effect of prostatic fluid.

It was also shown in this study that 33.3% of Enterococcus were from Pediatric ward whereas Wisplinghoff et al has demonstrated only 9% Enterococci from Pediatric ward[101]. Louis B. Rice et al in their study proved that relative proportion of enterococcus infection had increased in some instances to even more than 40%[41]. This sudden increase might be due to the sudden emergence of antibiotic resistant Enterococci in hospitals especially in pediatric ward.

In this study, it was observed that Enterococcus was commonly isolated in blood (55.5%) and the same was supported by A.Bedini et al who had reported in their study an incidence of 42.9% enterococci in blood stream infections[1]. S.Stefani et al also had documented that 41.3% of bacteremic cases were due to enterococcus[82].Patrick Murray had explained that most of the enterococcal bacteremias were of nosocomial origin because the enterococcus has the character of showing multiple drug resistance especially due to heavy use of antimicrobial agents[66].

It was also observed in this study that 77.7% of Enterococcus isolated were from inpatients. This in accordance with the study by Martinez Odriozola et al who had reported that 68% of enterococcal isolates were hospital accquired[51] and Patterson et al who had reported that 61% of enterococcal infections were nosocomial[67]. The period of study was post monsoon period in which many diarrhoeal cases were reported in the pediatric ward. Obviously, enterococci, a commensal of Gatrointestinal tract would have occured as a nosocomial agent in the pediatric ward where neonates with poor immunity and heavy doses of irrelevant antibiotics got admitted.

Species wise distribution of enterococcus showed that 50% of enterococcal isolates were E.faecium . It was noted in this study that 75% of E.faecium were from the paediatric ward and 90% of E. faecium in the pediatric ward were in the age group 0-1 month and 71.4 % pediatric cases were in males. This is in accordance with the study by Lata Kapoor etal who had also isolated 66% of E.faecium from paediatric cases from males[39]. Prematurity, low birth weight, increased number of days of hospitalisation , treatment via central venous line, parentral nutrition and antibiotic abuse might have attributed to the increased colonisation of neonates by enterococcus.

Specimen wise distribution showed that Blood was the most common specimen from which it was isolated (38.8%).Similar study by M.G Karmarkar etal have reported 80.7% of cases due to E.faecium[45]. Uma Chaudhary et al have stated that E.faecalis was the most common species in all clinical specimen except in blood where E.faecium was the most common isolate(50%)[96]. Mohanty et al in their study recovered 42.9% of E.faecium[60], which was the predominant isolate from blood wheras E.faecalis was mostly isolated from urine and pus.Thus the above studies correlated well with the present study.. The increasing predominance of E. faecium as a cause of bloodstream infections may partly reflect the high rate of antimicrobial resistance in this species . E.faecium more commonly acquires resistance to ampicillin and glycopeptides relative to other enterococcal isolates.Since ampicillin was one of the common antibiotic used in our hospital during the study period, the increased incidence of enterococcus faecium during that period was justified.

The present study on antibiotic susceptibility pattern of Enterococcus species showed that E.faecium was resistant to common antibiotics like Ampicillin (94.4%), Gentamycin (66.6%),Ciprofloxacin (72.2%), Doxycycline (61.1%), Erythromycin (66.6%) , Nitrofurantoin (72.2%) and Vancomycin (5.5%).This is in accordance with the study by Ujjala et al who showed E.faecium to have resistance to Gentamycin (77.7%), Ciprofloxacin (81%) and Erythromycin(86%)[96] but resistance to Ampicillin was only 54%. M.G. Karmarkar etal have documented 85.5% resistance to Nitrofurantoin , 100% resistance to Gentamycin and 28.57% resistance to Vancomycin[45]. All isolates showed 100% resistance to Cefotaxime and Ceftriaxone to which enterococci are intrinsically resistant .During the period of study, the four antibiotics used in the pediatric ward were Ampicillin, Gentamycin , third generation cephalosporins and Vancomycin. All these antibiotics were given intravenously through the infusion lines in their lower extremities either twice a day or three times a day. Obviously the isolates were resistant to most of the antimicrobials as well as to third generation cephalosprins which were already proved to be a resistant drug. Because of the indiscriminate use of antibiotics in the pediatric ward during the study period which was post monsoon and epidemics of diarrhoea were very common, the emergence of antibiotic resistant E. faecium in this ward was very obvious and justified.

SUMMARY

The study on the speciation of Enterococci in varied infections of GRH revealed that out of 200 samples from varied infections at GRH, 42% were urine samples and 39.5 % were blood samples. Among them, 52% were gram positive organisms from the pediatric ward in blood samples. Of the gram positive organisms, 50.7% were enterococcus isolates and 33.3% among them were from pediatric wards. Males predominated in 55% of the isolates and females only in 45%. More than 50% enterococcus were found in males in all wards except urology where only females showed more than 50%. Enterococci were isolated in 55% of the blood samples, 33.3% of them were from pediatric ward, 27.7% in the age group 0-1 month , 58.3% showed male predominance and 77.7 % were inpatients. The enterococci on speciation showed that 50% were E. faecium. Analysis of E.species showed that 38.8% were from blood, 75% were from pediatric ward, 90% were in the age group 0-1 month and 71.4 % were males. The enterococcus faecium was resistant to 94.4% ampicillin, 66.6% gentamycin and 100% ceftriaxone and 5.5% vancomycin. These four antibiotics were the common antibiotics used in the pediatric ward during the study perid. As the study period was post monsoon when a lot of diarrhoeal cases got admitted in the hospital, and the patients were administered with resistant antibiotics, E. faecium would have ocurred as a nosocomial pathogen.

CONCLUSION

- Enterococcal infections contributed to a significant proportion of infection in the population under study.
- The most common species was E.faecium.
- The patients infected with E.faecium were mostly children between 0-1 months and septicemia was the most common infection.
- Antimicrobial Susceptibility patterns revealed that the organism had developed resistance to the four antibiotics used during the study period in the wards.

Therefore this study has revealed that Enterococci could emerge as a significant agent of nosocomial infections especially in neonatalogy wards contributing to significant morbidity and mortality by virtue of Multi Drug Resistance.

ANNEXURE 1

1. GRAM STAINING:

The Gram stain was prepared as follows:

PRIMARY DYE:

Crystal violet	-	10 g
Ammonium Oxalate	-	4.25 g
Absolute alcohol	-	50 ml
Distilled water	-	500 ml

The methyl violet dye was dissolved in 50 ml absolute alcohol and mixed thoroughly. Then Ammonium oxalate 4.25 g was dissolved in 100 ml of Distilled water and this mixture was added to the violet stain and finally distilled water was added to make 500 ml. The total mixture was filtered before use.

Grams iodine solution consists of the following

Iodine	-	25 g
KI	-	50g
DW	-	500ml

Fifty grams of KI was dissolved in 500 ml of water and then 25 gms of I_2 was added to that. When iodine is dissolved, the solution was made upto 500 ml with Distilled water.

Counter stain used in Grams stain was dilute carbol fuchsin. It consists of the following:

Basic fuschin (powder)	5g
Phenol (Crystalline)	25g
Alcohol (95% of 100% Ethanol)	50ml

The basic fuschin powder was added to alcohol at intervals until it was fully dissolved. Then phenol too was dissolved in distilled water. Both the solution were mixed in a separate container.

2. CATALASE TEST:

Done by both slide & tube methods.

Tube method:

A small amount of the culture was picked up from the nutrient agar plate with a clean, sterile glass rod and inserted into a tube of 3% hydrogen peroxide, there was no effervescence or bubbles formation.

Slide method :

Pure growth of the organism from the agar was transferred to a clean slide with a sterile glass rod. Immediately 2 to 3 drops of 3% hydrogen peroxide was added to the growth, observed for the release of the bubbles.

3. BILE ESCULIN HYDROLYSIS TEST

This reaction presumptively identifies Group D Streptococci

Meat extract	3 gms
Peptone	5 gms
Ox bile purified and dehydrated	1 gm
Ferric ammonium citrate	.5 gm
NaCl	5 gm
Agar	15 gm
Distilled water	1 litre

Using the above ingredients, bile esculin plates were prepared.

The culture was inoculated onto the plate and incubated at 37^0C for 48 hrs. Enterococci and group D Streptococci produced blackening of the agar slant.

4. SALT TOLERANCE TEST

This is based on the ability of the Enterococci to grow in the presence of 6.5% NaCl incorporated into broth, while other Group D Streptococci are negative for this test. 6.5% NaCl broth was prepared with the following:-

Heart infusion broth	25g
Sodium chloride	65 gm
Indicator (1.6 g of bromothymol	
Blue in 100ml of 95% ethanol)	1 ml
Glucose	1 gm
Distilled Water	1 Litre

Dispensed into tubes of 5ml volume, autoclaved at 121^o C for 15 minutes. 2 to 3 colonies were inoculated into the broth, incubated at 35^o C overnight in ambient air incubator. It showed turbidity with color change of the medium from blue to yellow because of acid production by glucose fermentation.

5. HEAT RESISTANCE TEST

This is based on the principle that Enterococci can withstand heating at 60^o C for 30 minutes. This differentiates Enterococci from other Streptococci.

For this test, the isolated colonies 2 or 3 were inoculated into glucose broth, incubated at 37^o C overnight. The growth was judged with the turbidity, sub cultured from the broth onto one half on a nutrient agar plate, then the broth was placed in a water bath set at 60^o C for 30 minutes. Sub culture was done from the broth on the other half of the nutrient agar medium. After incubation at 37° C for 24 hrs, growth was observed on both halves of the plate, which indicates the heat resistant property of Enterococci.

6. SUGAR FERMENTATION TEST:

Todd Hewitt broth with bromothymol blue as indicator was used. Each tube was inoculated with 2 drops of an 18-24 hrs Todd Hewitt broth culture, incubated at 37° C in ambient air for 24-48 hrs. The tube was observed for color change from blue to yellow.

The following 1 % sterile sugars were used;

- Arabinose
- Raffinose
- Sucrose
- Sorbitol
- Mannitol

7. PYRUVATE FERMENTATION

With the following ingredients the pyruvate broth was prepared:-

Tryptone	10g
Yeast extract	5g
K_2HPO_4	5g
NaCl	5g
Sodium pyruvate	10g
Bromothymol blue	0.1 g
Distilled Water	1 Litre

pH adjusted to 7.2, sterilised at 121°C for 15 minutes. Fresh culture was inoculated into pyruvate broth, incubated at 37°C for 24-48 hours. Change in color from blue to yellow showed fermentation of pyruvate.

8. ARGININE DIHYDROLASE TEST

Arginine is first converted to citrulline by means of dihydrolase, then undergoes decarboxylation to form putrescine.

Moller’s arginine decarboxylase medium was prepared as follows:

Peptone	5g
Beef Extract	5g
Bromocresol Purple	0.01g
Cresol Red	0.005g
Pyridoxal	0.005g
Glucose	0.5g
Distilled Water	1 Litre

The solids were dissolved in water and the pH was adjusted to 6.0 before the addition of the indicators. To this is added 1 % L-arginine hydrochloride. Readjusted the pH to 6.0. Distributed 1 ml quantities in small tubes, autoclaved at 121^{o} C for 15 minutes.

Well isolated colonies were inoculated into two tubes of Moller’s decarboxylase medium, one with the amino acid and another devoid of amino acid as a control tube, overlaid both tubes with sterile mineral oil to 1cm and incubated at 37^{o}C for 4 days.

Conversion of the control tube to yellow color indicated that the organism is viable and pH has been lowered to activate the decarboxylase enzyme. Change in color of the medium from yellow to purple indicates amino acid has been deaminated to ammonia resulting in alkalinisation. Negative reaction was medium remaining yellow. The control tube also remained yellow.

9. MOTILITY BY

i) ***Hanging drop method***

ii) ***Semisolid motility medium(Mannitol motility medium)***

Using mannitol medium with 0.2% agar and 1% mannitol, organism stabbed once to half depth in tubes with straight wire. Examined after 18-24hours of incubation at 37ºC, for motility. Motile bacteria showing diffuse, hazy growth throughout the medium and non-motile bacteria growing only in the stabline, sharply defined margins leaving the surrounding medium clear. Further fermentation of mannitol to form acid also could be detected.

10 . HICROME ENTEROCOCUS AGAR BASE:

Hicrome Enterococcus faecium agar purchased from Himedia was used for the chromogenic differentiation of Enterococcus .

Ingredients	Grams/Litre
Peptone, special	23.0
Corn starch	1.0
Sodium chloride	5.0
Chromogenic sbstrate	0.1
Arabinose	10.0
Phenol red	0.1
Agar	15.0

Final pH (at 25ºC) 7.8 $\pm$ 0.2

E. faecalis produced blue colour colonies

E. faecium produced green coloured colonies with the surrounding medium turning yellow in colour due to arabinose fermentation.

PROFORMA

CASE HISTORY

Name : **Address:**

Age :

Sex :

Occupation :

Education :

Income :

IP No. :

Ward No. :

Diagnosis :

Date of admission :

Date of discharge :

Complaints :

Fever : Continuous, intermittent, low grade,
High grade, associated with chills / sweating.

Cough : Productive/nonproductive,diurnal variation

Sputum : Colour, purulent, non purulent, foul smelling, blood stained.

Pus : Colour, discharge – watery, purulent, blood stained.

Present history :

Past history : H/O DM, HT, Anaemia, Jaundice, Convulsions.

GENERAL EXAMINATION :

SYSTEMIC EXAMINATION :

CVS :

RS :

Per abdomen :

CNS :

TREATMENT DETAILS :

Antibiotics / Immuno Suppressives / Any Interventions/ Surgery

OUTCOME : **Cured / Improved/ Worsened / death.**

MICROBIOLOGICAL REPORT :

Specimen : **PUS/Blood/Sputum/CSF/Urine/Other Body Fluids**

Lab number :

Date :

Time :

TESTS DONE

A. Direct smear :

B. Culture :

Nutrient Agar :

MacConkey Agar :

Blood Agar :

Bile Esculin Agar :

Catalase Test :

Hanging drop :

6.5% Nacl Agar :

Heat Test :

TESTS FOR SPECIATION

Sucrose Fermentation :

Mannitol Fermentation :

Arabinose Fermentation :

Raffinose Fermentation :

Sorbitol Fermentation :

Pyruvate Fermentation :

HiChrome Enterococcus faecium Agar :

Pottassium Tellurite Agar :

Pigmentation :

ANTIBIOGRAM :

FINAL REPORT :

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GRAM STAIN

MANNITOL MOTILITY MEDIUM

CATALASE TEST

CULTURE

CULTURE

SALT TOLERANCE TEST

PIGMENTATION TEST

HEAT TEST

CHROMOGENIC AGAR

BIOCHEMICAL TESTS – E. faecalis

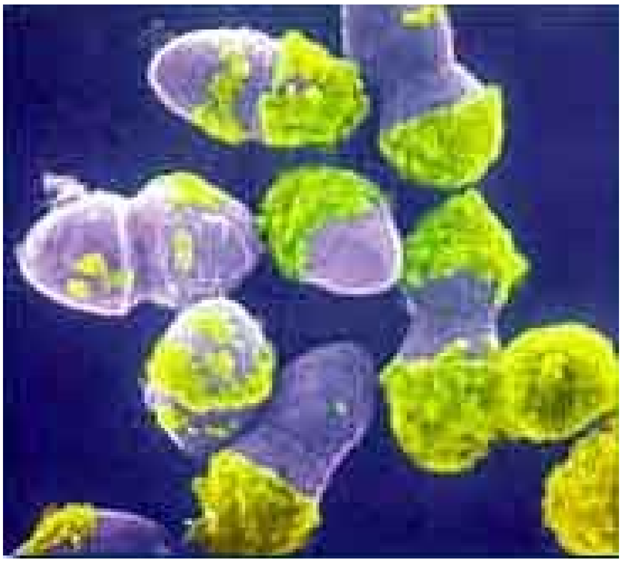
BLOOD TELLURITE AGAR

BIOCHEMICAL TEST – E.faecium

BIOCHEMICAL REACTIONS – E.durans

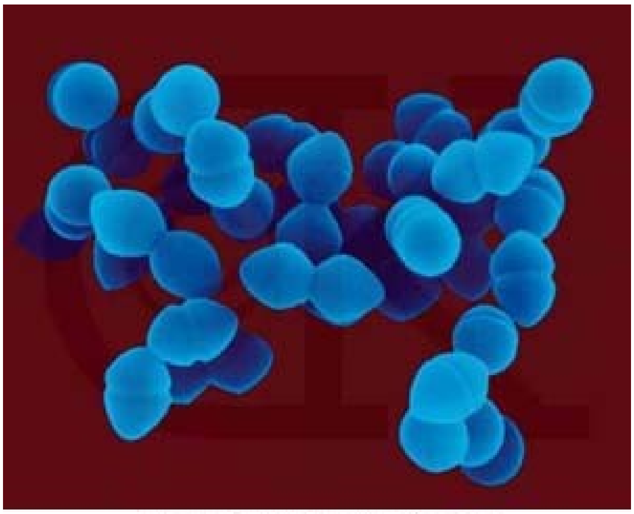
ANTIBIOGRAM

VRE

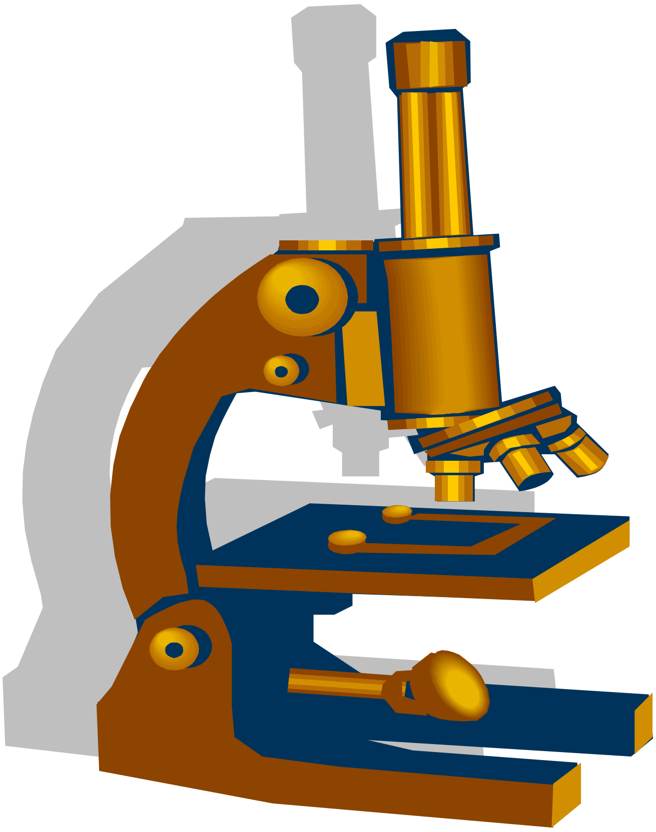


INTRODUCTION

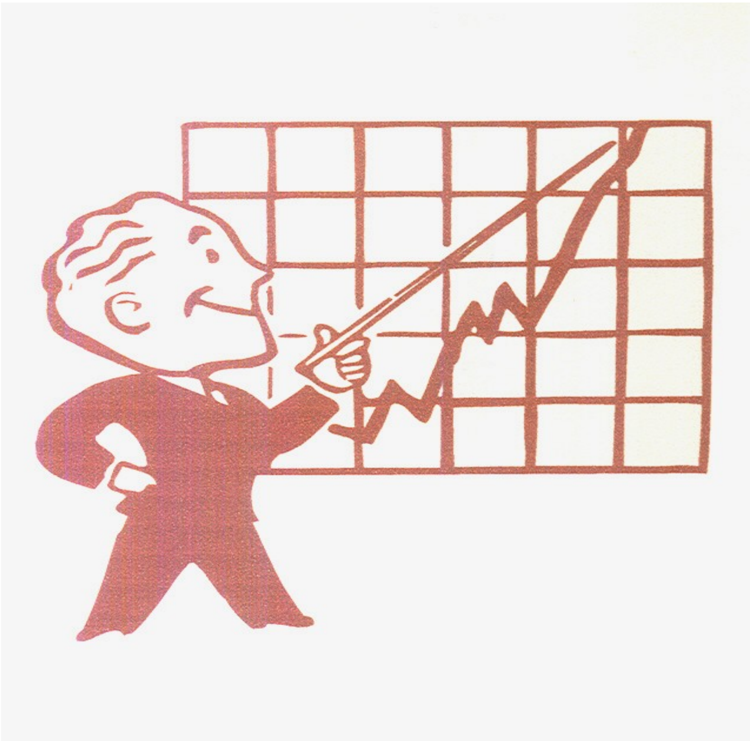
AIM AND OBJECTIVES



REVIEW OF LITERATURE



MATERIALS AND METHODS

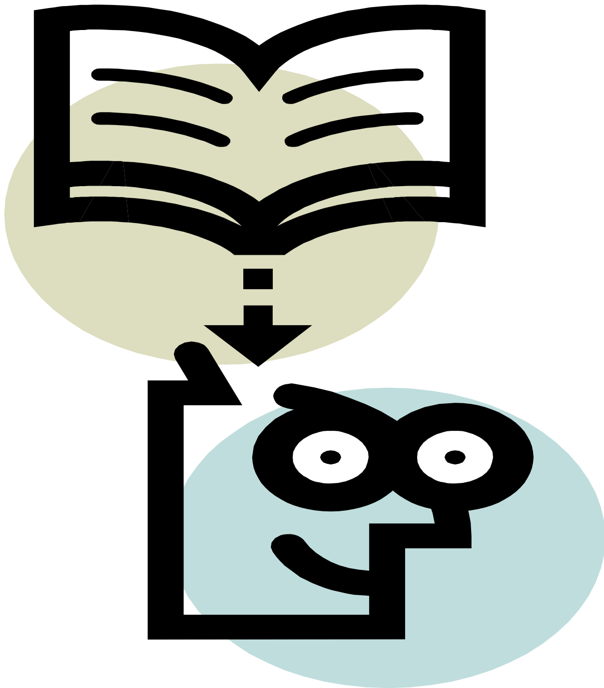


RESULTS

DISCUSSION

SUMMARY

CONCLUSION



BIBLIOGRAPHY

PROFORMA